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Complement Receptor Positive Spleen Cells in Microwave (2450-MHz)-Irradiated Mice

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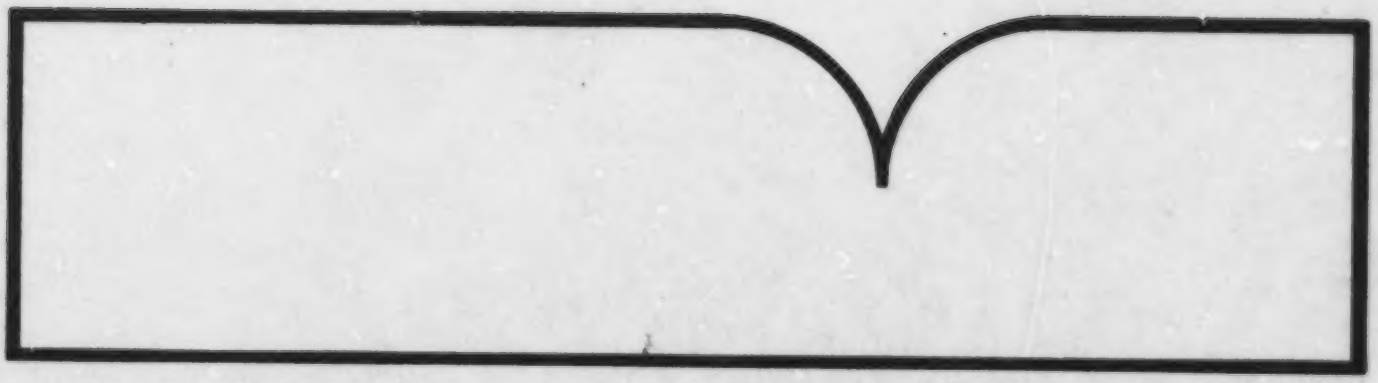

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16. ABSTRACT

Male CBA/J mice were exposed under far-field conditions in a temperature and humidity controlled environment to 2450-MHz (CW) microwaves. Mice were exposed once for 30 minutes at a power density of 15, 20, 30, or 40 mW/mc^2. The whole-body-averaged dose rate was approximately 0.7 mW/g per mW/cm^2. Six days after irradiation, the percentage of complement-receptor-positive (CR^+) spleen cells was determined. No difference was observed in the percentage of CR^+ spleen cells of young adult (10-12 week-old) mice exposed at any of the power densities as compared with sham-irradiated controls. However, a significant ($P<.05$) increase was observed in the percentage of CR^+ cells from 16-week old mice exposed at 40 mW/cm^2. This increase in CR^+ cells was accompanied by a significant ($P<.05$) decrease in the number of nucleated cells in the spleens of these mice. This change in CR^+ and nucleated spleen cells was not consistently produced. The available data indicate that the age and strain of the mouse, the microwave exposure characteristics, and the environmental conditions may all be sources of variation that affect the CR^+ end point.

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Complement Receptor Positive Spleen Cells in Microwave (2450-MHz)-Irradiated Mice*

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ABSTRACT

Male CBA/J mice were exposed under far-field conditions in a temperature and humidity controlled environment to 2450-MHz (CW) microwaves. Mice were exposed once for 30 minutes at a power density of 15, 20, 30, or 40 mW/cm². The whole-body-averaged dose rate was approximately 0.7 mW/g per mW/cm². Six days after irradiation, the percentage of complement-receptor-positive (CR^+) spleen cells was determined. No difference was observed in the percentage of CR^+ spleen cells of young adult (10-12-week-old) mice exposed at any of the power densities as compared with sham-irradiated controls. However, a significant ($P<.05$) increase was observed in the percentage of CR^+ cells from 16-week old mice exposed at 40 mW/cm². This increase in CR^+ cells was accompanied by a significant ($P<.05$) decrease in the number of nucleated cells in the spleens of these mice. This change in CR^+ and nucleated spleen cells was not consistently produced. The available data indicate that the age and strain of the mouse, the microwave exposure characteristics, and the environmental conditions may all be sources of variation that affect the CR^+ end point.

INTRODUCTION

Several studies have been reported that deal with effects of microwave radiation on the immune system (see the review by *Smialowicz*, 1979). These studies have been motivated primarily by a concern for possible adverse effects of microwaves on human beings. A debate has persisted in the scientific community as to the mechanism(s) by which microwaves could cause adverse effects. In some instances a thermal (measurable elevation of body temperature) burden on the exposed animal has been demonstrated to be associated with the observed changes (*Liburdy*, 1979, 1980), while in others a "non-thermal" (lack of measurable thermalization) or direct (athermal or field-specific effects unrelated to any detectable temperature change) interaction of microwaves with the immune system has been suggested (*Baranski* and *Czerski*, 1976). The large majority of reports of microwave-induced changes in the immune system lie somewhere between these two extremes, i.e., effects have been reported in the absence of measurable increases in body or tissue temperature, but at intensities that elicit thermoregulatory compensation for the thermal load (*Smialowicz et al.*, 1980a,b). Final interpretation of microwave-induced immunological effects obviously requires close scrutiny of exposure parameters and of experimental design and methodologies. Equally important is the corroboration of a reported effect by independent investigators. This last criterion, however, has seldom been met in studies of biological effects of microwaves. This paper deals with an attempt to corroborate a microwave-induced immunological effect.

Wiktor-Jedrzejczak et al. (1977a,b) reported an increase in the percentage of complement-receptor-positive (CR^+) cells in the spleen of mice six days after a single exposure for 30 min to 2450-MHz microwaves at an average whole body dose rate of approximately 14 mW/g. We were unable to corroborate this effect in earlier work (*Smialowicz et al.*, 1979). In this earlier work, we hypothesized that failure to corroborate might have been due to differences in microwave exposure parameters. We exposed mice to 2450-MHz microwave under far-field conditions in an anechoic chamber, whereas *Wiktor-Jedrzejczak et al.* (1977a,b) used a TE_{10}

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waveguide-exposure system. We also considered that our failure to produce an increase in CR^+ spleen cells may have been due to our use of a different strain of mouse (BALB/c) from that (CBA/J) used by *Wiktor-Jedrzejczak et al.* (1977a,b). The strain-specificity alternative took a more prominent position as a possible source of explanation when it was learned that CBA/J but not BALB/c mice display an increase in CR^+ cells following microwave irradiation (*Schlagel et al.*, 1979, and from *C. Schlagel*, 1979, personal communication). More recently, further work on microwave-induced increases in CR^+ cells has been reported from that laboratory (*Sulek et al.*, 1980). In light of this new information, we have again attempted to corroborate the effects originally reported by *Wiktor-Jedrzejczak et al.* (1977a,b). We report the results of exposure of CBA/J mice to microwaves (2450-MHz) and the determination of the percentage and number of CR^+ spleen cells in these mice six days after a single 30-minute exposure.

MATERIALS AND METHODS

Mice

Male CBA/J mice (Jackson Lab., Bar Harbor, ME) were received in our vivarium at 8 weeks of age. The mice were allowed to acclimate to their new surroundings for at least 2 weeks prior to experimentation. Unless stated otherwise, mice were used for microwave experiments at 10–12 weeks of age. The mice were maintained on a 12:12 light-dark cycle and were allowed food and water *ad libitum* while in the home cage.

Microwave Exposure System

Irradiation was conducted in a 2450-MHz exposure facility in which temperature, humidity, and air flow were controlled (*Elder* and *Ali*, 1975). The microwave field was generated by a Varian Model PPS-2.5 AS industrial heating unit at a frequency of 2450 MHz in a continuous-wave mode. A total root-mean-squared (RMS) ripple of 11% was measured after the installation of a full-wave bridge in the filament circuit. A controlled-environment exposure chamber (61 x 76 x 76 cm) constructed of foamed polystyrene was positioned beneath a 10-dB-gain pyramidal-horn antenna in the far field.

The environmental chamber was confined in an electrically anechoic chamber that was lined with microwave-absorbing material (Model CBV24 and VCVB24, Emerson and Cuming, Inc., Canton, MA). The environmental-control system permitted regulation of the temperature and humidity in the exposure chamber and in an identical foamed-polystyrene sham-irradiation chamber located outside of the anechoic chamber. The air temperature was maintained at 24 ± 0.5°C, relative humidity at 50 ± 10%, the averaged lineal velocity passing through the chambers was 0.31 ± 0.07 m/s for all microwave and sham-irradiation exposures.

Animal Exposures

Mice confined individually in vented-polystyrene cages, 6 cm on edge, were placed in one of four positions of a diamond-shaped array, and were spaced equidistant to each other (19 cm) and 9.5 cm from the center of the microwave-exposure chamber. The volume of the polyethylene cage allowed the mouse freedom of movement during exposure. Age-matched mice of the same strain and sex were positioned in a similar array in the sham-irradiation chamber. Mice were irradiated for 30 min at power densities of 15, 20, 30, or 40 mW/cm^2, which would respectively result in specific-absorption (dose) rates (as determined by twin-well calorimetry) of approximately 11, 14, 22, and 29 mW/g (*Smialowicz et al.*, 1979). Following exposure, mice were immediately returned to their home cages.

Complement Receptor Lymphocytes

The number of complement-receptor-positive (CR^+) cells in mouse spleens was determined as described by *Bianco et al.* (1970). Six days following microwave- or sham-irradiation, mice were killed by cervical dislocation. Their spleens were immediately removed under aseptic conditions. Spleens were minced and erythrocytes were lysed using Tris-Buffered NH_4Cl, as described by *Boyle* (1968). Spleen-cell suspensions were washed twice with RPMI-1640 medium with 25 mM HEPES (Grand Island Biological Co., Grand Island, NY) containing 100 units/mℓ penicillin, 100 μg/mℓ streptomycin, and 5% heat-inactivated fetal-calf serum. Spleen cells were suspended to 2 x 10^6 viable (as determined by Trypan blue exclusion) cells/mℓ in RPMI medium containing 0.01 M EDTA. One-tenth mℓ of this cell suspension was incubated with 0.1 mℓ of EAC reagent at 37°C for 30 min; the EAC reagent consisted of sheep erythrocytes (E), the 19S

fraction of rabbit anti-sheep erythrocyte antibody (A) from Cordis Laboratory, Miami, Fl, and fresh mouse serum, which served as a source of complement (C). Following incubation, the cells were gently resuspended and 50 $\mu\ell$ of this suspension was added to an equal volume of a 1% solution of brilliant cresyl blue in saline. The number of lymphocytes that formed rosettes (three or more sheep erythrocytes adhering per lymphocyte) was determined. A total of 300 mononuclear cells from duplicate preparations were counted, and the percentage of CR^+ cells was determined. Results were analyzed by Student's t test.

RESULTS AND DISCUSSION

The percentage of CR^+ spleen cells in 10-12-week old mice 6 days after the single 30-min exposure to 2450-MHz is shown in Table 1. Irrespective of power density, no significant difference was observed in the percentage of CR^+ cells in the spleens of microwave-exposed mice as compared with sham-irradiated controls. No significant difference was observed in the number of nucleated cells in the spleens from microwave-exposed mice as compared with sham-irradiated controls at any of the power densities employed.

The mice that were irradiated at 40 mW/cm^2 were visibly under thermal stress after the microwave exposure. Their fur was ruffled and wet, a sign that the mice had coated their fur with urine or saliva in an attempt to cool themselves. That these mice were obviously thermally stressed was demonstrated in another experiment in which 6 of 25 mice died during or soon after a single 60-min exposure at 40 mW/cm^2. Mice exposed at 30, 20, or 15 mW/cm^2 did not display the signs of thermal stress.

Wiktor-Jedrzeczak et al. (1977a,b) observed an increase in CR^+ spleen cells 6 days following microwave exposure at an average absorbed dose rate approximating 14 mW/g. The mice exposed in our study at 20 mW/cm^2 received virtually an identical whole-body averaged dose. Power densities of 15 and 30 mW/cm^2, and absorption rates of 11 and 22 mW/g, respectively, are within or very near the range of microwave dose rates required to cause an increase in CR^+ cells as recently reported by *Sulek et al.* (1980). Our failure to observe a change in CR^+ cells led us to another consideration. We learned that the age of the mice exposed to microwaves may be a critical factor in inducing an increase in CR^+ spleen cells, older mice being more responsive and retired breeders being the most responsive (*Schlagel*, personal communication). Consequently, we looked at the effect that age might have on microwave-induced changes in CR^+ cells.

Table 1 Percentage of CR^+ spleen cells in male CBA/J mice six days after a single 30-min exposure to 2450-MHz microwaves at different power densities.

Power density (mW/cm^2)	Mean percent CR^+ Cells ± SE		P
	Sham[a]	Exposed	
15	32.7 ± 1.0	32.2 ± 1.0	NS[b]
20	34.1 ± 1.0	32.9 ± 0.4	NS
30	32.5 ± 0.6	32.0 ± 0.3	NS
40	32.4 ± 0.7	32.5 ± 0.6	NS

[a]Eight sham and 8 exposed mice used at each power density. All mice 10–12 weeks of age.
[b]Not significant, i.e. $P > .05$, Student's t test.

Table 2 shows the results of experiments in which 16-week-old mice were exposed to microwaves. When the older mice were exposed at 40 mW/cm^2, a significant ($P < .05$) increase in CR^+ cells was observed in their spleens six days after irradiation. However, a significant ($P < .05$) decrease in the number of nucleated cells in the spleens was observed for these same animals as compared with sham-irradiated controls. An attempt was made to repeat this finding in 14-week old mice and retired breeders (24 weeks old). No difference, however, was observed in either the percentage of CR^+ cells or in the spleen cellularity of microwave-irradiated compared with sham-irradiated mice from either group (Table 2). Retired breeders exposed at 30 mW/cm^2 also did not show any change in CR^+ cells or spleen cellularity as compared with sham-irradiated controls (Table 2).

Table 2 The effect of age on the percentage of CR^+ spleen cells in male CBA/J mice six days after a single 30-min exposure to 2450-MHz microwaves.

Power density (mW/cm^2)	Mice (N)	Age (weeks)	Mean ± SE	
			% CR^+	Cells/spleen ($x10^7$)
40	Sham (8)	16	29.8 ± 0.3	6.35 ± 0.44
	Exposed (8)	16	34.6 ± 0.8[a]	5.03 ± 0.41[a]
40	Sham (6)	14	33.3 ± 0.9	6.53 ± 0.69
	Exposed (6)	14	33.8 ± 1.1	6.32 ± 0.69
40	Sham (6)	24[b]	30.8 ± 1.6	11.5 ± 1.43
	Exposed (8)	24	34.6 ± 2.1	13.0 ± 0.78
30	Sham (8)	24[b]	32.8 ± 1.5	9.05 ± 1.92
	Exposed (8)	24	35.3 ± 0.7	10.9 ± 2.44

[a]$P < .05$, Student's t test
[b]Retired breeders

The exposure of young adult mice to 2450-MHz under far-field conditions in an anechoic chamber does not lead to a consistent increase in CR^+ spleen cells 6 days following irradiation. The only time that a change in CR^+ cells occurred was when 16-week old mice were exposed at 40 mW/cm^2. In this instance, a significant decrease was also observed in the cellularity of spleens from these mice. The increase in CR^+ cells in this case may be due to a redistribution of CR^+ cells to the spleen or to a shift in the spleen-cell populations, which might be due to a decrease of cortisone-sensitive T-lymphocytes. Cortisone is cytotoxic for certain populations of T-lymphocytes (*Claman*, 1972). Mice exposed at 40 mW/cm^2 experienced microwave-induced thermal stress, which is known to cause an increase in adrenal cortical steroids in the blood (*Lotz* and *Michaelson*, 1978; *Liburdy*, 1979). Also, thermogenic levels of microwave radiation have been shown to affect the distribution of lymphocytes in mice (*Liburdy*, 1980).

A change in CR^+ spleen cells in 16-week-old mice was observed after a 40 mW/cm^2 exposure, but not at lower power densities. It appears that a significant degree of microwave-induced thermal stress is required to produce this effect under the conditions described here. On the other hand, *Wiktor-Jedrzejczak et al.* (1977a,b) and *Sulek et al.* (1980) reported an effect at absorption levels that elicited little or no increase in core temperature as compared with sham-exposed mice. This does not, however, mean that thermalization of tissues by microwaves was not responsible for the effect. We have demonstrated that microwaves (2450-MHz) at an absorption rate of 3.5 mW/g can cause a significant increase of colonic temperature in CBA/J mice with drug-induced hypothermia (*Smialowicz et al.*, 1980). Untreated mice were capable of maintaining normothermia by active thermoregulation even at an average dose rate of 7.2 mW/g despite the added thermal burden.

It is difficult to reconcile differences of data from the two laboratories. The most obvious procedural difference lies in the microwave-exposure apparatus employed in the two laboratories and in the orientation of animals in the field. In our earlier work (*Smialowicz et al.*, 1979), we indicated that the orientation of the mouse in the waveguide was important since *Wiktor-Jedrzejczak et al.* (1977c) reported that a pronounced increase in CR^+ cells occurred when the long axis of the mouse was oriented perpendicular to the cross sectional area of the waveguide, whereas increased variability resulted when the mouse was oriented parallel to the cross sectional area. We suggested (*Smialowicz et al.*, 1979) that deposition of microwave energy might cause thermal "hot spots" to develop in critical tissues because of orientation in a waveguide. This hypothesis is less tenable since other investigators (*Sulek et al.*, 1980) have recently reported that the orientation of mice in a waveguide during exposure had no effect on the induction of increases in CR^+ cells. This leaves us with the impression that the energy deposition in mice exposed dorsally in the far-field must be quite different from that of mice exposed in a waveguide. Determination of the pattern of energy deposition in mice exposed under these different conditions may explain the difference in results. Consequently, further work is needed to corroborate this microwave-induced increases in CR^+ spleen cells and to delineate the variables responsible for the increase.

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DISCLAIMER

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